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The administration of pyridostigmine bromide (PB), permethrin (Perm) and N,N, diethyl-m-toluamide (DEET) as single agents, or in combination, may result in neurobehavioral toxicity and an altered immune response which may differ between male and female subjects. The behavior of male and female rats was studied in experiments designed to measure various aspects of central nervous system functioning in the presence of subtoxic doses of PB, Perm and DEET. PB administration altered locomotor activity in male and female rats, but more so in females than in males. PB, Perm and DEET decreased fixed-interval and fixed-ratio response rates in male rats, but there was no evidence for synergism when drug combinations were tested. Acute and repeated PB administration delayed response acquisition in male and female rats. As measured in human lymphocytes, Perm and DEET showed immunosuppression in different cell proliferation assays, in contrast to PB which showed minimal immunosuppression. Perm and DEET combinations showed greater immunomodulatory activity than PERM/PB and DEET/PB combinations.

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INTRODUCTION

Some of the 650,000 male and 50,000 female U.S. soldiers who served during the Gulf War were exposed to prophylactic doses of the cholinesterase inhibitor pyridostigmine bromide (PB) possibly in combination with pesticides such as permethrin (PERM) and insect repellents such as N,N,-Diethyl-M-Toluamide (DEET). Very little information is available concerning the neurobehavioral and immunological toxicity of these compounds, but it has been hypothesized that this exposure may have contributed toward symptoms associated with the 'Gulf War Syndrome'.

The main hypothesis of this multidisciplinary research effort is that the administration of PB, PERM and DEET as single agents, or in combination, results in neurobehavioral toxicity and an altered immune response which may differ between male and female subjects. To evaluate this hypothesis, the behavior of adult male and female rats will be studied in experiments designed to measure various aspects of CNS functioning in the presence of sub-toxic doses of PB, PERM, and DEET. The neurobehavioral analyses are complemented by an assessment of the immune response in rats and lymphocytes from healthy human volunteers.

BACKGROUND

It has been suggested that exposure to PB, PERM and DEET may have played a role in the development of the syndrome which appears to have afflicted some of the military personnel who served during the Gulf War (Almog, et al., 1991; Clem, et al., 1993). PB is a quartenary ammonium compound that is classified as an anticholinesterase agent. It inhibits the hydrolysis of acetylcholine (Ach) by competitive reversible binding to acetylcholinesterase. PB decreases nerve gas toxicity by occupying acetylcholinesterase binding sites. Although PB and nerve gas share the same mechanism of action, PB is much less toxic due to the reversible binding and the short duration of action. During the Gulf War, PB was taken prophylactically when there was a high risk of exposure to nerve gas. The pyrethrins, of which PERM is a synthetic example, are considered to exhibit low acute toxicity since they are rapidly hydrolyzed in the gastrointestinal tract following oral ingestion and by liver esterases in the blood (Metcalf and McKelvey, 1974). Hydrolysis of PERM results in the production of pyrethrin, and pyrethroid alcoholic, phenolic or carboxylic acid metabolites which are excreted as the glycine, sulfate, glucuronide or glucoside conjugates (Czasida, et al., 1983; Miyamoto, 1976). In sufficient concentrations however, pyrethrins have been shown to be neurotoxic, with effects including hypersensitivity, tremors and seizures (Dorman and Beasley, 1991). During the Gulf War this compound was used to impregnate some battle-dress uniforms in the field. DEET is the most commonly used insect repellent in the world (Veltri, et al. 1994). It is the active ingredient in such products as Deep Woods Off. It has also been proposed as a pharmaceutical excipient to improve dermal and transdermal delivery of drugs (Windheuser, et al., 1982). The exact mechanism of DEET toxicity is unknown, but pathological findings indicate that it is a demyelinating agent which causes spongiform myelinopathy (Verschoyle, et al, 1992). DEET was available during the Gulf War, but used infrequently. PB, PERM and DEET have all been used as individual agents with

an apparent low rate of adverse events. Some recent evidence suggests, however, that the neurotoxicological effects of combinations of these agents may exceed their individual effects. McCain (1995) has reported that large oral doses of PB, PERM and DEET kill male laboratory rats, either when the compounds are administered simultaneously, or when PB is administered together with PERM or DEET. The effects were greater than additive, except when DEET and PERM were administered concurrently. It is not known whether similar results would have been obtained in female subjects, but there are reasons to assume that the neurobehavioral effects of these different compounds may be gender-dependent (Barbarino, et al., 1991; O'Keane and Dinan, 1992).

LOCOMOTOR ACTIVITY

THE EFFECTS OF PYRIDOSTIGMINE BROMIDE, PERMETHRIN AND DEET ALONE, OR IN COMBINATION, ON LOCOMOTOR ACTIVITY AND THIGMOTAXIS IN MALE AND FEMALE RATS (workplan year 1 and 2).

Changes in locomotor activity are an important source of information to the behavioral toxicologist as they provide a first indication of a compound's behavioral effects when administered in sub-toxic doses.

PYRIDOSTIGMINE BROMIDE AFFECTS OPEN FIELD SPEED AND CENTER TIME IN MALE AND FEMALE RATS. J.B. Hoy, F. van Haaren, I.R. Tebett and J.L. Karlix (abstract # 589.1, presented at the Annual Meeting of the Society for Neuroscience, New Orleans, 1997).

Pyridostigmine bromide (PB) was used by a large number of soldiers during the Persian Gulf War as an oral prophylactic against nerve-gas poisoning, and PB has been suggested as a cause of Gulf War Syndrome. Experimentally naive male and female (pro- and metestrus) Sprague Dawley rats were tested to determine the effects of PB on open field locomotion rate and thigmotatic response (center time). Following PB doses of 0, 3, 10 or 30 mg/kg by gavage, rats were videotaped in a m^2 arena for 2 hours beginning 30 minutes post-treatment. Videotapes were analyzed using Dynamic Animal Movement Analyzer software to determine speed and center time in 20 min bins. Drug uptake into the blood of the rats was chromatographically confirmed.

Analysis of mean speeds and center times using a 2 factor mixed design revealed a significant interaction of sex, dose, and by group. PB doses of 10 and 30 mg/kg reduced male speeds 21% and 38% respectively. Females: 34% and 60% respectively. Male center times were similarly affected, with reductions of 10% and 57% respectively. Females: reductions of 34% and 65% respectively. In general, speed and center time were dependent on dose and females were more sensitive to PB than males.

PYRIDOSTIGMINE BROMIDE ALTERS LOCOMOTION AND THIGMOTAXIS OF RATS: GENDER EFFECTS. J.B. Hoy, B.A. Cody, J.L. Karlix, C.J. Schmidt, I.R. Tebbett, S. Toffolo, F. can Haaren and D. Wielbo (submitted to Pharmacology Biochemistry and Behavior).

Pyridostigmine bromide may be a factor contributing to Gulf War Syndrome. Male rats and female rats in the pro-estrus and met-estrus stages of estrus were tested to determine the effects of pyridostigmine bromide on locomotion rate and thigmotactic response using doses of 3.0, 10.0, and 30.0 mg/kg. Thirty minutes after administration of the pyridostigmine bromide the rats were video-recorded for 2 h in a 1 m^2 open field arena. The rat's activities were anlayzed for the drug's effect on speed throughout the 2 h and during six 20 min segments. Also, the times that the rats were observed moving through the central 50% of the arena were determined. Locomotion rates decreased significantly and thigmotaxses increased significantly in all groups of rats as a dose response to pyridostigmine bromide. Habituation occurred over 2 h for both responses, primarily during the first 40 minutes. Female rats were more affected than males, but met-estrus and pro-estrus females did not differ significantly in their responses. At the highest dose the effect was persistent from 30 minutes to 150 minutes post-treatment. Pro-estrus females dosed at 30 mg/kg had much higher pyridostigmine bromide serum levels than met-estrus females and males. Implications for humans are discussed.

LEARNING and MOTIVATION

THE EFFECTS OF PYRIDOSTIGMINE BROMIDE, PERMETHRIN AND DEET, ALONE OR IN COMBINATION ON LEARNING AND PERFORMANCE IN MALE AND FEMALE SPRAGUE-DAWLEY RATS (workplan year 1,2).

It has frequently been shown that the effects of drugs and toxins not only depend on their chemical properties, but also on the baseline against which their behavioral effects are established (van Haaren, 1992a, 1993). The better known example is that of psychomotor stimulant drugs such as cocaine: its administration tends to increase low response rates, and decrease high response rates (van Haaren, 1992a). The present experiment was designed to measure the effects of PB, PERM, and DEET, alone or in combination on behavior maintained by a multiple fixed interval 2-min, fixed ratio 50 (MULT FI 2 min, FR 50) schedule of reinforcement. FI schedules maintain much lower performance rates than FR schedules and this arrangement allows us to investigate whether or not workload alters the behavioral effects of small dose of PB, PERM and DEET alone, or in combination, in male and female rats.

THE EFFECTS OF PYRIDOSTIGMINE BROMIDE, PERMETHRIN AND DEET ALONE, OR IN COMBINATION, ON FIXED-RATIO AND FIXED- INTERVAL BEHAVIOR IN MALE SPRAGUE-DAWLEY RATS F. van Haaren. B.A. Cody, S. Haworth, J.B. Hoy, J.L. Karlix, I.R. Tebbett, C.R. Schmidt and D. Wielbo (Second Annual Meeting of Federally-funded Gulf War Investigators, Washington D.C., 1998).

The present experiment was designed to evaluate the behavioral effects of small doses of pyridostigmine bromide (PB), permethrin (PERM) and N,N, diethyl toluamide (DEET) on well-established schedule-controlled behavior. Male Sprague-Dawley were trained to respond on a multiple fixed-ratio 50, fixed-interval 2-min (MULT FR 50, FI 2g) schedule of reinforcement. They were then challenged with different doses of PB (-30 min, 0, 3, 10 or 30 mg/kg, by gavage 5 ml/kg), PERM (-15 min, 0, 15, 30 or 60 mg/kg, intraperitoneally, 2 ml/kg), or DEET (-30 min, 0, 50, 200 or 500 mg/kg, by gavage, undiluted). The low and medium doses of PB did not affect FR or FI response rates, but the high dose of PB resulted in a significant decrease in response rates during both schedule components. The low and medium doses of PERM did not affect FR or FI response rates either, but the high dose of PERM (60 mg/kg) decreased response rates in both components of the schedule. DEET administration had little effects on response rates maintained by the FR and FI schedules, a small decrease in responding was observed after the administration of the highest dose (500 mg/kg). Following the completion of the dose-effects curves for the individual compounds subjects were challenged with different drug combinations to determine whether or not synergistic interactions would be observed. Subjects were thus challenged with PB 1.5 mg/kg in combination with either 7.5 mg/kg PERM or 25 mg/kg DEET and PB 5 mg/kg in combination with either 15 mg/kg PERM or 100 mg/kg DEET. Synergistic drug interactions were not observed in the context of the present experimental parameters.

Other experiments were designed to assess the extent to which repeated administration of small doses of pyridostigmine bromide, alone or in combination with other chemicals, interferes with the acquisition of a novel response. This particular paradigm was selected to be an additional part of our behavioral test battery because previous experiments have shown that it is sensitive to manipulation of behavioral and pharmacological variables (e.g. LeSage, et al., 1996; van Haaren, 1992b).

THE EFFECTS OF ACUTE AND REPEATED PYRIDOSTIGMINE BROMIDE ADMINISTRATION ON RESPONSE ACQUISITION WITH IMMEDIATE AND DELAYED REINFORCEMENT. F. van Haaren, R. de Jongh, J.B. Hoy, J.L. Karlix, C. J. Schmidt, I.R. Tebbett and D. Wielbo (submitted to Pharmacology Biochemistry and Behavior, 1998)

This experiment was designed to assess the effects of acute and repeated administration of the Gulf War chemical pyridostigmine bromide on response acquisition. Experimentally naïve, male Sprague-Dawley rats were exposed to a situation in which lever presses were either immediately followed by pellet presentation or after a 16-s resetting delay. Different groups of rats received either one acute administration of pyridostigmine bromide (10 mg/kg, by gavage) or repeated pyridostigmine administration for seven days (1.5 mg/kg /day, by gavage). Other groups were treated with distilled water for the same period of time. Both acute and repeated pyridostigmine bromide administration decreased serum cholinesterase levels by approximately 50%, but neither treatment affected brain cholinesterase levels. Acute and repeated drug administration produced the same behavioral effects. Subjects exposed to the 0-s delay conditions obtained many more food pellets that those exposed to the 16-s delay conditions. Administration of pyridostigmine bromide delayed the onset of responding in some, but not all, of the subjects in the treated groups independent of the delay condition to which they were exposed. Many more responses were observed on an inoperative lever during the 16-s delay conditions than during the 0-s delay conditions, especially during the 16-s delay condition in which subjects had received acute vehicle administration. Whether or not these effects of small doses of pyridostigmine bromide on response acquisition are of central or peripheral origin will need to be determined in future studies, as response acquisition in the present experiment may have been affected by pyridostigmine's effects on gastro-intestinal functioning and/or motor activity.

THE EFFECTS OF THE GULF WAR CHEMICAL PYRIDOSTIGMINE BROMIDE AND PERMETHRIN, ALONE OR IN COMBINATION, ON RESPONSE ACQUISITION IN MALE AND FEMALE RATS. F. van Haaren,, B.A. Cody, J.B. Hoy, J.L. Karlix, C.R. Schmidt, I.R. Tebbett and D. Wielbo (manuscript in preparation, 1998).

Concurrent exposure to the carbamate cholinesterase inhibitor pyridostigmine bromide and the insecticide permethrin may have contributed to the development of the Gulf War Syndrome. The present experiment was designed to investigate to what extent these compounds when administered alone, or in combination, interfere with learning in male and female rats. Different groups of subjects were treated with pyridostigmine bromide (1.5 mg / kg / day, by gavage) or distilled water, once a day for seven consecutive days (approximate Gulf War dose). Subjects then also received an intraperitoneal injection of permethrin (vehicle, 15 or 60 mg/kg) before the experimental session in which they had an opportunity to earn food pellets by pressing a lever. Baseline cholinesterase activity in female rats was higher than that in male rats. Repeated pyridostigmine administration decreased cholinesterase activity in male and female rats compared to baseline control levels. The low dose of permethrin further decreased cholinesterase activity in male and female rats in the presence of pyridostigmine bromide, but increased cholinesterase activity in females in the presence of distilled water. The high dose of permethrin further decreased cholinesterase activity in male and female rats in the presence of both pyridostigmine bromide and distilled water. Serum concentrations of permethrin in rats

repeatedly treated with pyridostigmine bromide exceeded those in rats treated with distilled water and they were higher in female rats than in males. Pyridostigmine bromide also consistently increased body temperature in male rats which was dose-dependently decreased by permethrin. Food-restricted male and female rats treated with distilled water and the permethrin vehicle quickly learned to contact a lever when lever contacts were followed by food pellet presentation. Pyridostigmine bromide decreased such responding in male and female rats. Permethrin increased responding on the operative lever in male rats, but further decreased reinforced responding in female rats. Permethrin administration increased responding in the non-reinforced lever in both male and female rats. The results of the experiment are discussed in terms of the peripheral and central effects of pyridostigmine bromide and permethrin when administered alone, or in combination.

IMMUNOLOGY

THE IMMUNOMODULATORY EFFECTS OF PYRIDOSTIGMINE BROMIDE, DEET AND PERMETHRIN (workplan year 1,2).

The exact etiology and pathophysiology of the Gulf War syndrome are poorly understood. War syndromes from the Civil War to the Gulf War have been described which have complicated the understanding of the illness associated with the Gulf War. Some of the 720,000 soldiers who served during the Gulf War were exposed to prophylactic doses of the cholinesterase inhibitor, pyridostigmine bromide possibly in combination with pesticides such as permethrin and insect repellents such as N,N-diethyl-M-Toluamide. Very little information is available on the immunotoxicological effects of these compounds.

Many of the signs and symptoms of the Gulf War Syndrome are similar to those of chronic fatigue syndrome including joint pain, fatigue and depression. Like the Gulf War Syndrome [GWS], the etiology and pathophysiology of chronic fatigue syndrome is unclear. One of the primary causes of chronic fatigue syndrome is hypothesized to be focussed upon immune dysfunction (Downey, 1992; Holmwood and Shannon, 1992; Murdoch, 1992; Blondel-Hill and Shafran, 1993). Because the GWS signs and symptoms are so similar to those reported in chronic fatigue syndrome, this study was undertaken to evaluate the immunotoxicological effects of Pyridostigmine bromide (PB), N,N,-diethyl-m-toluamide (DEET) and permethrin (PERM).

THE IMMUNOMODULATORY EFFECTS OF PYRIDOSTIGMINE, PERMETHRIN AND DEET. J.L. Karlix, B. Freiburger, J.B. Hoy, F. van Haaren, I.R. Tebbett, D. Wielbo, C. Schmidt. (Second Annual Meeting of Federally-funded Gulf War Investigators, Washington D.C., 1998).

The Gulf War Syndrome is a complex group of signs and symptoms which has plagued thousands of veterans who served during the Persian War. The exact etiology is unclear, however clinical presentation is often similar to the chronic fatigue syndrome which may contain an immunopathophysiological component. Therefore, this study was undertaken to investigate whether certain chemicals that soldiers were exposed to during the Gulf War possess any immunomodulatory effects. Human lymphocytes were isolated and exposed to varying concentrations of permethrin [perm], pyridostigmine [Pb] and DEET. The human lymphocytes were stimulated via mitogens PMA [phorbol-12-myristate 13-actetate], PHA [phytohemagglutinin], and MLR [mixed lymphocyte response] and immune response was measured either as immunostimulation or immunosuppression. All three agents demonstrated a dose-dependent response. Perm and DEET showed the greatest immunomodulatory activity with statistical differences against controls in the PMA, PHA and MLR as measured by cpm. Perm IC50's were 4.8 ug/ml PMA, 7.5 PHA and 46 ug/ml MLR. DEET was not as potent as Perm with IC50's of 100 ug/ml PMA, 95 ug/ml PHA and 50 ug/ml MLR. In contrast to the other agents, PB did not reach an IC50 but showed immunostimulation at low concentrations. All three agents demonstrated immunomodulatory effects that must be considered when addressing the pathophysiology of the Gulf War Syndrome.

THE SYNERGISTIC IMMUNOMODULATORY EFFECTS OF THE CHEMICALS USED DURING THE GULF WAR. J.L. Karlix, B. Freiburger, J.B. Hoy, F. van Haaren, I.R. Tebbett, D. Wielbo and C.R. Schmidt (manuscript in preparation, 1998).

The Gulf War Syndrome is a complex group of signs and symptoms which has plagued thousands of veterans who served during the Persian War. Recently our laboratory discovered that several of the agents produced immunomodulatory effects. Therefore, this study was undertaken to investigate whether the combination of Gulf War chemicals that soldiers were exposed to during the Gulf War possessed any immunomodulatory effects. Human lymphocytes were isolated and exposed to varying combinations of permethrin [perm], pyridostigmine [Pb] and DEET to evaluate for synergism. The human lymphocytes were stimulated via mitogens PMA [phorbol-12-myristate 13-actetate], PHA [phytohemagglutinin], and MLR [mixed lymphocyte response] and immune response was measured either as immunostimulation or immunosuppression.

The combination of Perm and DEET demonstrated the greatest immunomodulatory activity in comparison with combinations of Perm/PB and DEET/PB. Only Perm/DEET showed statistical significance against controls in the PMA, PHA and MLR as measured by cpm and reached an

IC50 of DEET 50/PERM 30 mcg/ml. However, all three different combinations [Perm/DEET, PB/DEET, PB/PERM] showed slight immunostimulation at concentrations below 10 mcg/ml suggesting some potential for excess antibody production. Although there was slight immune modulation in all three different combinations, Perm/DEET had the greatest effect on lymphocyte function. Because of the immunomodulation seen in the combination of these agents, immunotoxicological effects should be considered as a potential mechanism of toxicity for these chemicals.

THE IMMUNOMODULATORY EFFECTS OF CHRONIC EXPOSURE TO GWS CHEMICALS. J.L. Karlix, J.B. Hoy, F. van Haaren, I.R. Tebbett, P. Rabiansky and C.R. Schmidt (manuscript in preparation, 1998).

The immunodulatory effects of chronic exposure to GWS chemicals were assessed in rat lymphocytes isolated from rat spleens. Our preliminary data shows that in the interleukin 2/calcium dependent pathways stimulated by PHA, females exhibited a slight immunostimulatory effect in the pyridostigmine rats. This finding supports the hypothesis that the GWS may in fact be due to an autoimmune clinical picture that is similar to the chronic fatigue syndrome. However, this was not consistent in the PMA or protein kinase C stimulated rat lymphocytes that showed a suppression of immune response. In contrast, the male rats showed no effect in the PHA experiments and an immunostimulatory effect in the PMA tests. These preliminary results suggest that pyridostigmine chronic exposure modulates the rat immune response that may be a contributing factor to the GWS pathophysiology.

REFERENCES

Almog S, E Winkler, Y Amitay, S Dani, M Shefi, M Tirosh & J Shemer (1991). Acute pyridostigmine overdose: a report of nine cases. *Israel Journal of Medical Science*, 27, 659-663.

Barbarino A, SM Corsello, A Tofani, R Sciuto, S Della Casa, CA Rota & A Barini (1991). Sexual dimorphism of pyridostigmine potentiation of growth hormone (GH)-releasing hormone-induced GH release in humans. *Journal of Clinical Endocrinology and Metabolism*, 73, 75-78.

Blondel-Hill E & SD Shafran (1993). Treatment of the chronic fatigue syndrome. A review and practical guide. *Drugs*, 46(4): 639-51

Czasida JE, DW Gammon, AH Glickman & JW Lawrence (1983). Mechanisms of selective action of pyrethroid insecticides. *Annual Review of Pharmacology and Toxicology*, 23, 413-438.

Dorman DC & VR Beasley (1991). Neurotoxicology of pyrethrin and the pyrethroid insecticides. *Veterinary and Human Toxicology*, 33, 238-243.

Downey DC (1992). Fatigue syndromes: new thoughts and reinterpretation of previous data. *Medical Hypotheses*, 39(2): 185-90.

Holmwood C & C Shannon (1992). Chronic fatigue syndrome. A review from the general practice perspective. *Aust-Fam-Physician*, 21(3): 278-9, 283-5.

LeSage MG, T Byrne & A Poling (1996). Effects of d-amphetamine on response acquisition with immediate and delayed reinforcement. *Journal of the Experimental Analysis of Behavior*, 66, 349-367.

McCain WC (1995). Acute oral toxicity study of pyridostigmine bromide, permethrin and DEET in the laboratory rat. Study 75-48-2665. U.S. Army Center for Health Promotion and Preventive Medicine.

Metcalf RL & JJ McKelvey (Eds.) (1974). The future for Insecticides. John Wiley & Sons New York.

Miyamoto J (1976). Degradation, metabolism and toxicity of synthetic pyrethroids. *Environmental Health Perspectives*, 14, 15-28.

Murdoch JC (1992). Chronic fatigue syndrome. A review from the general practice perspective. *Aust-Fam-Physician*, 21(8): 1205-6.

O'Keane V & TG Dinan (1992). Sex steroid priming effects of growth hormone response to pyridostigmine throughout the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism,* 75, 11-14.

van Haaren F (1992a). Differential effects of cocaine on high and low response rates maintained with and without rate requirements. *Behavioural Pharmacology*, 3, 435-441.

van Haaren F (1992b). Response acquisition with fixed and variable resetting delays of reinforcement in male and female Wistar rats. *Physiology and Behavior*, 52, 767-772.

van Haaren F (1993). Schedule-controlled behavior: positive reinforcement. In: van Haaren F (ed.) *Methods in Behavioral Pharmacology*, Elsevier Science, New York, pp. 81-99.

Veltri JC, TG Osimitz, DC Bradford et al. (1994). Retrospective analysis of calls to poison control center resulting from exposure to the insect repellant n,n-diethyl-m-toluamide (deet) from 1985-1989. *Clinical Toxicology, 32, 1-16.*

Verschoyle RD, AW Brown, C Nolan, DE Ray & T Lester (1992). Acomparison of the acute toxicity, neuropathology, and electrophysiology of N,N-diethyl-m-toluamide and N,N-dimethyl-2,2,-diphenylacetamide in rats. *Fundamental and Applied Toxicology*, 18, 79-88.

Windheuser JJ, JL Haslam, L Caldwell & RD Shaffer (1982). The use of N,N-diethyl-m-toluamide to enhance dermal and transdermal delivery of drugs. *Journal of Pharmaceutical Sciences*, 71, 1211-1213.

PUBLICATIONS and PRESENTATIONS

Hoy JB, F van Haaren, IR Tebbett & JL Karlix (1997). Pyridostigmine bromide affects open-field speed and center time in male and female rats. Annual Meeting of the Society for Neuroscience, New Orleans, LA.

Hoy JB, BA Cody, JL Karlix, CJ Schmidt, IR Tebbett, S Toffolo, F van Haaren & D Wielbo. Pyridostigmine bromide alters locomotion and thigmotaxis of rats: gender effects. (submitted to Pharmacology Biochemistry and Behavior).

Karlix JL, B Freiburger, JB Hoy, F van Haaren, IR Tebbett, D Wielbo & CR Schmidt (1998). The immunomodulatory effects of pyridostigmine, permethrin and DEET. Second Annual Meeting of Federally-funded Gulf War Investigators, Washington D.C.

Karlix JL, B Freiburger, JB Hoy, F van Haaren, IR Tebbett, D Wielbo & CR Schmidt. The synergistic immunomodulatory effects of the chemicals used during the Gulf War. (manuscript in preparation).

Karlix JL, JB Hoy, F van Haaren, IR Tebbett, P Rabiansky & CR Schmidt. The immunomodulatory effects of chronic expsoure to GWS chemicals. (manuscript in preparation).

van Haaren F, JB Hoy, JL Karlix & IR Tebbett (1997). Gulf War Illness: effects of pyridostigmine bromide and permethrin alone, or in combination, on response acquisition in male Sprague-Dawley rats. Annual Meeting of the Behavioral Toxicology Society, Palm Beach, FL.

van Haaren F, BA Cody, S Haworth, JB Hoy, JL Karlix, IR Tebbett, CR Schmidt & D Wielbo (1998). The effects of pyridostigmine bromide, permethrin and DEET alone, or in combination, on fixed-ratio and fixed-interval behavior in male Sprague-dawley rats. Second Annual Meeting of Federally-funded Gulf War Investigators, Washington D.C.

van Haaren F, R de Jongh, JB Hoy, JL Karlix, CJ Schmidt, IR Tebbett & D Wielbo. The effects of acute and repeated pyridostigmine bromide administration on response acquisition with immediate and delayed reinforcement. (submitted to Pharmacology Biochemistry and Behavior)

van Haaren F, BA Cody, JB Hoy, JL Karlix, CR Schmidt, IR Tebbett & D Wielbo. The effects of the Gulf War chemicals pyridostigmine bromide and permethrin alone, or in combination, on response acquisition in male and female rats. (manuscript in preparation).